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Genetic Diversity within *Cryptosporidium parvum* and Related *Cryptosporidium* Species

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To assess the genetic diversity in *Cryptosporidium parvum*, we have sequenced the small subunit (SSU) rRNA gene of seven *Cryptosporidium* spp., various isolates of *C. parvum* from eight hosts, and a *Cryptosporidium* isolate from a desert monitor. Phylogenetic analysis of the SSU rRNA sequences confirmed the multispecies nature of the genus *Cryptosporidium*, with at least four distinct species (*C. parvum*, *C. baileyi*, *C. muris*, and *C. serpentis*). Other species previously defined by biologic characteristics, including *C. wrairi*, *C. meleagridis*, and *C. felis*, and the desert monitor isolate, clustered together or within *C. parvum*. Extensive genetic diversities were present among *C. parvum* isolates from humans, calves, pigs, dogs, mice, ferrets, marsupials, and a monkey. In general, specific genotypes were associated with specific host species. A PCR-restriction fragment length polymorphism technique previously developed by us could differentiate most *Cryptosporidium* spp. and *C. parvum* genotypes, but sequence analysis of the PCR product was needed to differentiate *C. wrairi* and *C. meleagridis* from some of the *C. parvum* genotypes. These results indicate a need for revision in the taxonomy and assessment of the zoonotic potential of some animal *C. parvum* isolates.

Cryptosporidiosis is a coccidian infection of humans, domestic animals, and other vertebrates. More than 20 *Cryptosporidium* species have been described in various animal hosts (30). The validity of most species, however, has not been established, because cross-transmission studies indicate that some isolates of *Cryptosporidium* are infective to several animal species (31). Only six to eight species (*C. parvum*, *C. wrairi*, *C. felis*, and *C. muris* in mammals, *C. baileyi* and *C. meleagridis* in birds, *C. serpentis* in reptiles, and *C. nasorum* in fish) are considered valid *Cryptosporidium* species by most researchers (15, 30). The validity of these six or eight species has been questioned recently by another group of researchers because a genetic analysis failed to support their classification as different species (41).

Because *C. parvum* is generally considered to be the parasite responsible for infection in most mammals, efforts have been made to examine the biologic and molecular diversity of this parasite. Although *C. parvum* isolates from humans, farm animals, companion animals, and rodents are morphologically and developmentally similar, differences in host specificity, prepatent and patent periods, and pathogenicity have been observed. For example, many isolates from humans are not infective for calves, mice, or guinea pigs. In contrast, bovine isolates are infective for humans, neonatal calves, and mice (32). Similarly, although isolates from wild, adult house mice can easily infect wild, uninfected adult mice (22), *C. parvum* bovine isolates are infective only for neonatal mice (35, 40). Differences in host specificity have been used previously as the basis for identifying *C. wrairi* and *C. felis* as unique species (11, 19). It remains to be determined how these biologic differences in other isolates relate to species differentiation.

Other than genetic differences between human and bovine isolates of *C. parvum* oocysts (5–7, 25, 26, 32, 38, 39), the inter- and intraspecies biological differences in *Cryptosporidium* have been infrequently substantiated by molecular studies. We recently sequenced the complete small subunit (SSU) rRNA genes of various *Cryptosporidium* isolates and used them in a phylogenetic analysis (46). Results of our study have shown that *Cryptosporidium* parasites are a multispecies complex containing at least four species, *C. parvum*, *C. baileyi*, *C. muris*, and *C. serpentis. C. felis*, *C. nasorum* and *C. meleagridis* were not studied. Differences were also observed between human and bovine isolates. Recently, based on sequences from the acetyl-coenzyme A synthetase gene, the internal transcribed spacer of rRNA, and a 298-bp region of the SSU rRNA genes, several new genotypes (pig, mouse, and koala) have been identified (27, 28).

In the present study, we have extended the phylogenetic analysis to include *C. felis*, *C. meleagridis*, and some other *C. parvum* (dog, pig, kangaroo, ferret, mouse, and monkey) isolates. We have included *C. meleagridis* in this study because a recent diagnostic report suggested that *C. meleagridis* may be closely related to *C. parvum* (9). The objectives of the present study were to test observations on the multispecies nature of *Cryptosporidium* parasites and to determine if *C. parvum* is much more diverse than previously believed. Findings from this study may contribute to a rationale for the revision of the taxonomy of the genus *Cryptosporidium*.

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TABLE 1. Isolates of *Cryptosporidium* parasites used in this study[a]

Isolate	Species	Host	Location
HFL2	*C. parvum*	Human	Florida
HFL5	*C. parvum*	Human	Florida
CRPM1	*C. parvum*	Rhesus monkey	Georgia
BOH6	*C. parvum*	Calf	Ohio
CPF1	*C. parvum*	Ferret	Georgia
CPD1	*C. parvum*	Dog	Ohio
CPM1	*C. parvum*	Mouse	Maryland
P1	*C. parvum*	Pig	Australia
K2	*C. parvum*	Red Kangaroo (*Macropus rufus*)	Australia
GP1	*C. wrairi*	Guinea pig	Michigan
CMEL1	*C. meleagridis*	Turkey	North Carolina
C8	*C. felis*	Cat	Australia
CSP01	*C. serpentis*	Corn snake (*Elaphe g. guttata*)	Kansas
IDVS-811	*C. muris*	Cow	Idaho
CBA01	*C. baileyi*	Chicken	Alabama
CSP06	*Cryptosporidium* sp.	Desert monitor (*Varanus griseus*)	Missouri

[a] Full sequences were also obtained from 2 additional *C. parvum* bovine isolates, 1 human isolate, 2 *C. muris* isolates, and 3 *C. serpentis* isolates. Partial sequences covering the most polymorphic regions (about 820 bp) were also obtained from additional 48 *C. parvum* human genotype isolates, 20 bovine genotype isolates, 4 murine isolates, 3 dog isolates, 3 ferret isolates, 2 pig isolates, 2 koala isolates with the kangaroo sequence, 1 snake isolate with the desert monitor sequence, 5 *C. felis* isolates, 2 *C. meleagridis* isolates, and 2 *C. baileyi* isolates.

MATERIALS AND METHODS

***Cryptosporidium* isolates.** Isolates used in this study were from humans, cattle, dogs, cats, mice, pigs, turkeys, ferrets, a monkey, and a desert monitor. With the exception of *C. meleagridis*, which was originally isolated from a turkey and has been passaged in 1- to 2-week-old turkey pouts, all isolates were from naturally infected animals or humans and had no other identifiable parasites. *Cryptosporidium* species were determined by oocyst morphology, host origin, and traditional classification of *Cryptosporidium* parasites. Accordingly, the parasites with small-type oocysts from humans, calves, mice, ferrets, dogs, pigs, marsupials, and the monkey were all identified as *C. parvum* (15, 30, 33). Whenever possible, multiple isolates from the same host or closely related hosts were characterized to confirm the identity of parasites and the accuracy of data. To assess the relationship of these parasites to other *Cryptosporidium* parasites, sequences of *C. wrairi*, *C. baileyi*, *C. muris*, and *C. serpentis* previously obtained by us (46) were also used in the phylogenetic analysis. A complete list of isolates and sources is shown in Table 1.

Oocyst isolation and DNA extraction. Oocysts were purified from fecal samples by a combination of discontinuous density sucrose gradient centrifugation and isopycnic Percoll centrifugation or cesium chloride gradient centrifugation (1, 2). After treatment in 5.25% sodium hypochlorite solution (100% commercial bleach) at 4°C for 10 min, oocysts were washed five times by suspending in 10 ml of sterile water, centrifuging at 1,500 × *g* for 10 min, decanting supernatant, and resuspending in sterile water. DNA was extracted from purified oocysts by a previously published technique (21).

PCR and DNA sequencing. The entire SSU rRNA gene was amplified from samples by conventional polymerase chain reaction by using the forwarding primer 5′-AACCTGGTTGATCCTGCCAGTAGTC-3′ and reverse primer 5′-TGATCCTTCTGCAGGTTCACCTACG-3′. Each PCR consisted of 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 60 s, with an initial denaturation at 94°C for 5 min and a final extension at 72°C for 10 min. After PCR amplification, the PCR fragment was sequenced by using an ABI377 autosequencer. Sequence accuracy was confirmed by two-directional sequencing, by the sequence of a second PCR product, and in most cases (see footnote for Table 1), by sequencing of the most polymorphic regions (about 820 bp) of multiple isolates.

Sequence analyses. Multiple alignment of the DNA sequences was done with the Wisconsin package version 9.0 from Genetics Computer Group, Madison, Wisconsin, with manual adjustment. Two types of phylogenetic analysis were used on the aligned sequences to assess relationships among isolates, the distance-based neighbor-joining analysis and parsimony analysis. For the former, neighbor-joining trees (34) were constructed with the program TreeconW (42), based on the evolutionary distances between different isolates calculated by Kimura 2-parameter analysis. For the latter, a CONSENSE parsimony tree was made by using the phylogenetic analysis software PHYLIP version 3.5 (17). For neighbor-joining tree construction, an initial analysis used sequences from *Eimeria tenella* (GenBank accession no. AF026388) as an outgroup to assess the relationship among different *Cryptosporidium* spp. A subsequent analysis used *C. muris* and *C. serpentis* as the outgroup to assess the relatedness of isolates within the *C. parvum* group. Tree reliability was assessed by the bootstrap method (16) with 1,000 pseudoreplicates. We considered a value of 95% to be statistically significant (14); however, values above 50% are reported, since bootstrap may be a conservative estimate for the reliability of a clade (18). The multiple sequence alignment was deposited in GenBank and is retrievable by using the accession number for any sequence from this study.

PCR-RFLP analysis. We previously developed a PCR-restriction fragment length polymorphism (RFLP) technique for species and genotype-specific diagnosis of *Cryptosporidium* parasites (46). Because only three *C. parvum* genotypes (bovine, human, and *C. wrairi*) were used in the initial technical development, we evaluated the performance of this technique in differentiating various genotypes of *C. parvum*. A PCR product of about 1,325 bp was amplified first by primary PCR with primers 5′-TTCTAGAGCTAATACATGCG-3′ and 5′-CCCTAATCCTTCGAAACAGGA-3′. The PCR contained 10 μl of Perkin-Elmer (Norwalk, Conn.) 10× PCR buffer, 6 mM $MgCl_2$, 200 μM (each) deoxynucleoside triphosphate, 100 nM (each) primer, 2.5 U of *Taq* polymerase, and 0.25 to 1 μl of DNA template in a total 100-μl reaction mixture. A total of 35 cycles were carried out, each consisting of 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min, with an initial hot start at 94°C for 3 min and a final extension at 72°C for 7 min. A secondary PCR product of 826 to 864 bp (depending on isolates) was then amplified from 2 μl of the primary PCR with primers 5′-GGAAGGGTTGTATTTATTAGATAAAG-3′ and 5′-AAGGAGTAAGGAACAACCTCCA-3′. The PCR and cycling conditions were identical to primary PCR, except that 3 mM $MgCl_2$ was used in the PCR.

For restriction fragment analysis, 20 μl of the secondary PCR product was digested in a total of 50 μl of reaction mixture, consisting of 20 U of *Ssp*I (New England BioLabs, Beverly, Mass.) for species diagnosis or *Vsp*I (GIBCO BRL, Grand Island, N.Y.) for genotyping of *C. parvum* and 5 μl of respective restriction buffer at 37°C for 1 h, under conditions recommended by the supplier. The digested products were fractionated on 2.0% agarose gel and visualized by ethidium bromide staining.

Nucleotide sequence accession number. The nucleotide sequences of the SSU rRNA gene of *Cryptosporidium* parasites have been deposited in the GenBank database under accession no. AF093489 to AF093499, AF112569 to AF112576, AF115377, and AF115378.

RESULTS

In our previous study, we evaluated the species structure of the genus *Cryptosporidium* by using SSU rRNA gene sequences from *C. parvum* (from cattle and humans), *C. wrairi*, *C. baileyi*, *C. muris*, and *C. serpentis* and showed that *C. parvum*, *C. baileyi*, *C. muris*, and *C. serpentis* differed from each other at distances comparable to or greater than those among different species of apicomplexans (46). In the present study, we obtained complete SSU rRNA gene sequences from two additional *Cryptosporidium* species (*C. felis* and *C. meleagridis*), *C. parvum* isolates from mice, pigs, dogs, ferrets, a monkey, and a kangaroo, and an isolate from a desert monitor with small-type oocysts (4 to 5 μ). These sequences were used in more extensive phylogenetic analyses to evaluate the validity of *Cryptosporidium*

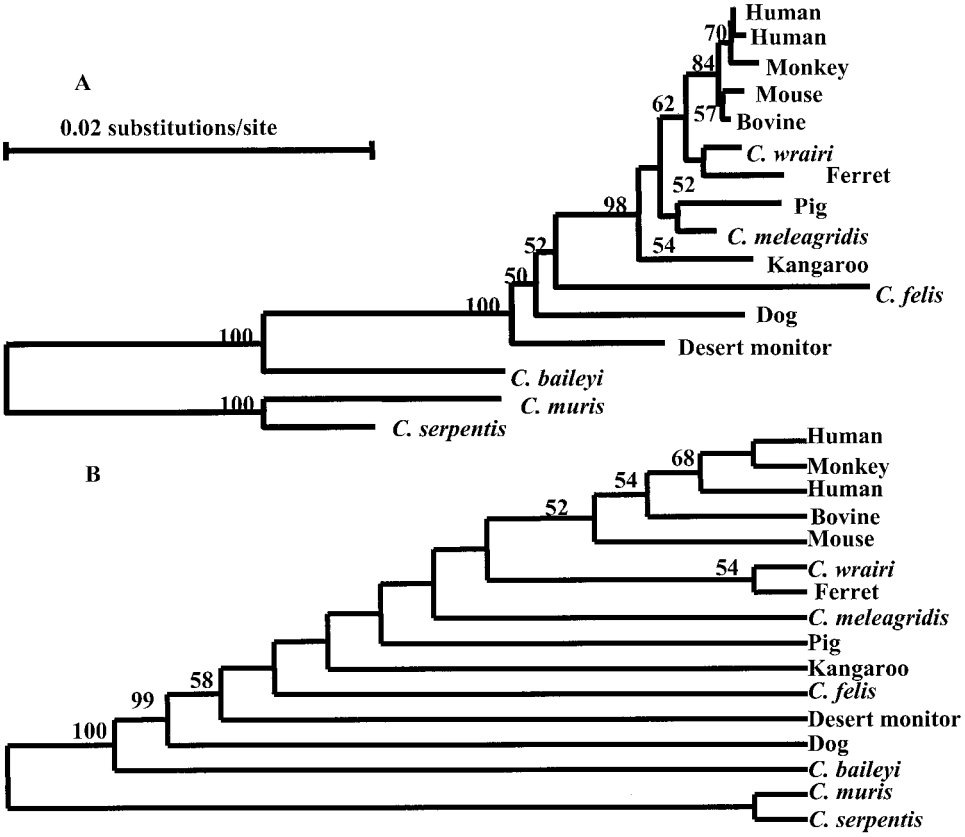

FIG. 1. Phylogenetic relationship of *Cryptosporidium* parasites within the broad *C. parvum* group by neighbor-joining (A) and parsimony (B) analyses. Bootstrap values above 50% from 1,000 pseudoreplicates are also shown.

speciation, relationships among various species, and genetic diversity within *C. parvum*.

Both the neighbor-joining and the parsimony methods were used in the phylogenetic analyses of the SSU rRNA gene of the seven *Cryptosporidium* species. In an initial neighbor-joining analysis, *E. tenella* was used as an outgroup. This analysis confirmed the previous observation that *Cryptosporidium* species formed two groups, with full statistical reliability. One group contained *C. muris* and *C. serpentis*. The other group contained *C. baileyi*, *C. felis*, *C. meleagridis*, *C. wrairi*, all *C. parvum* isolates, and the isolate from a desert monitor (data not shown). A second analysis was made with *C. muris* and *C. serpentis* as an outgroup to assess the relationship among *C. parvum*, *C. wrairi*, *C. meleagridis*, and *C. felis* (Fig. 1A). In both phylogenetic analyses, *C. baileyi* diverged from *C. parvum*, *C. meleagridis*, and *C. wrairi* (100% bootstrap). *C. wrairi* and *C. meleagridis*, however, clustered within the different isolates of *C. parvum. C. felis* was separated from the majority of *C. parvum* isolates, as was true for the isolate from a desert monitor. Parsimony analysis revealed similar phylogenetic relationships among different *Cryptosporidium* isolates, with *C. muris*, *C. serpentis*, and *C. baileyi* well separated from the rest (Fig. 1B).

Extensive diversity was observed in the large *C. parvum* cluster (Fig. 1). Neighbor-joining analysis showed that the human and monkey isolates of *C. parvum* formed a monophylatic clade, which originated from the same sources with another monophylatic clade of bovine and murine isolates (Fig. 1A). The isolate from the ferret clustered together with *C. wrairi*, forming a sister group to the human and bovine *C. parvum* group. A kangaroo isolate was distant from the majority of members in the *C. parvum* group, as was the parasite from dogs. The pig isolate, on the other hand, grouped together with *C. meleagridis*. The *Cryptosporidium* isolate from a desert monitor also clustered together the *C. parvum* group. A similar observation regarding genetic diversity within the broad *C. parvum* group was made by parsimony analysis (Fig. 1B). The relationship among different isolates, however, was less well defined, and the bootstrap values were lower.

SSU rRNA sequences unique to particular genotypes were identified in the *C. parvum* group (*C. parvum*, *C. wrairi*, *C. meleagridis*, *C. felis*, and the desert monitor isolate). There were eight genotypes of *C. parvum*, which differed from each other primarily in four areas of the SSU rRNA gene (Table 2). The closely related *Cryptosporidium* species, *C. wrairi*, *C. meleagridis*, *C. felis*, and the isolate from a desert monitor, also had unique nucleotide sequences in these four areas. *C. felis*, the *C. parvum* dog genotype, and the desert monitor isolate also differed from *C. parvum* in other areas of the SSU rRNA gene (data not shown). The majority of the differences within the *C. parvum* group, however, were found in the first half of the SSU rRNA gene.

Based on the SSU rRNA gene sequences from *C. parvum*, *C. baileyi*, *C. muris*, and *C. serpentis*, we previously developed a nested PCR-RFLP technique for species and genotype differentiation (46). Species diagnosis was made by digesting the secondary PCR product (826 to 864 bp) with *Ssp*I, and differentiation of *C. parvum* genotypes by digestion with *Vsp*I. In the present study, we evaluated the ability of this technique to differentiate extended members of the *C. parvum* group. Di-

TABLE 2. Differences among isolates in the *C. parvum* group (*C. parvum*, *C. wrairi*, *C. felis*, and *C. meleagridis*) in four areas of the SSU rRNA gene

Source or genotype	Location of mutations in the SSU rRNA gene[a]			
	182–189	273–286	639–656	689–699
Bovine	AAACTCGA	ATTAA------A	AAATATTTTGATGAATATT-----TATATAAT	ACTA-----------TATATTTTAGT
Human	AAACTCGA	AATTA------A	AAATATTTTGATGAATATT-----TATATAAT	ACTA---------TTTTTTTTTTTAGT
Monkey	AAACTCGA	AATTA------A	ATATATTTTGATGAATATT-----TATATAAT	ACTA----------TTTTTTTTTAGT
Ferret	AGGCCTGA	ATAAA------T	AAATATTTTGATTAATATT-----TATATAAT	ACTA---------AATTTTTGTTTGGT
Guinea pig[b]	AGGCCCGA	ATAAA------T	TAATATTTTGA-AAATATT-----TATATAAT	ACTA----------TATATTTTTAGT
Turkey[c]	AAACCTGA	AATTT------A	TAATA-TTTGATTAATATT-----TATATAAT	ACTA-----------AATTTATTAGT
Mouse	AAACTCGA	ATTAA------A	AAATATTTTAATTAATATT-----TATATAAT	ACTA--------TAATTATTTTTTAGT
Pig	AAACCTAA	ATTTTTA----A	TAATATTTT--T-AATATT-----TATATAAT	ACTA---------TAATTTTTATTAGT
Kangaroo	GA-CCTGA	ATAAATA----A	TTATACTTTTTAAGGTGTT-----TATATAAT	ACTA----------TATTTTTTTAGT
Lizard	AGGCCTGA	AATTAT-----T	TAATATTACG----GTATT-----TATATAAT	ACT----------TTATTTTTAGAGT
Dog	AAACCTGA	ATTTT------A	TAATATTT---AACATATT-----TATATAAT	ACTA--------------TTTATAGT
Cat[d]	GA-CCCTA	AATAATTTATTT	TAATATTTTTTTTTTAAATATTAATATGTAAG	TTTAAGACTGAATTTTTAGTTTTGATA
C. baileyi	AGACCCGA	ATTT-------A	CAATACCACG----GTATT-----TATATAAC	ACT-------------TATTTAAAGT

[a] Nucleotide positions in the aligned sequences of all *Cryptosporidium* species. Actual positions in individual sequences vary because of the introduction of gaps (dashes) in the aligned sequences (1,757 bp).
[b] *C. wrairi*.
[c] *C. meleagridis*.
[d] *C. felis*.

gestion of the secondary PCR products from the *C. parvum* group parasites with *Ssp*I resulted in four predicted restriction patterns. The *C. parvum* human, monkey, bovine, mouse, ferret, and kangaroo isolates, *C. wrairi*, and *C. meleagridis* showed an identical restriction pattern, with three visible bands of 109 to 112, 254, and 441 to 461 bp in size (Table 3). The *C. parvum* dog isolate and the desert monitor isolate also had a three-band pattern, but with a smaller (417- to 418-bp) upper band. In contrast, the *C. parvum* pig isolate and *C. felis* each had only two visible bands (365 and 453 bp and 390 and 426 bp, respectively) that were different in size from the two-band patterns of *C. baileyi* (254 and 572 bp), *C. muris* (385 and 448 bp), and *C. serpentis* (370 and 414 bp). Electrophoresis of the digested secondary products largely confirmed the predicted restriction patterns, except for that of the *C. parvum* kangaroo isolate, which consistently showed partial digestion of the upper band (Fig. 2). It was possible to differentiate by *Ssp*I digestion the *C. parvum* bovine genotype A gene from the B gene, which yielded a larger lower band (119 versus 108 bp) than the A gene (Fig. 2).

Digestion of the secondary PCR products from the *C. parvum* group parasites with *Vsp*I yielded three additional patterns as follows: (i) *C. parvum* human and monkey isolates; (ii) *C. parvum* bovine, dog, pig, and kangaroo isolates, *C. wrairi*, *C. felis*, and the desert monitor isolate; and (iii) *C. parvum* mouse and ferret isolates and *C. meleagridis*. Restriction digestion with *Ssp*I and *Vsp*I could differentiate the *C. parvum* human and monkey genotypes from all other genotypes, but DNA sequencing was needed to differentiate the *C. parvum* bovine genotype from the kangaroo genotype and *C. wrairi* (Table 2). Electrophoresis of digested products confirmed the predicted restriction patterns, with the exception of *C. felis* isolates, which consistently showed partial digestion by *Vsp*I (Fig. 2).

DISCUSSION

Results of this study confirm the heterogeneous nature of *Cryptosporidium* parasites. Based on SSU rRNA sequences, the genus *Cryptosporidium* contains at least four species: *C. muris*, *C. serpentis*, *C. baileyi*, and *C. parvum*. Several *Crypto-*

TABLE 3. RFLP (in base pairs) in the SSU rRNA gene of various *Cryptosporidium* spp. and genotypes

Species	Source	PCR fragment no.	*Ssp*I digestion[a]	*Vsp*I digestion[a]
C. muris	Cattle, camel, hyrax	833	**385, 448**	**102, 731**
C. serpentis	Snake	831	14, 33, **370, 414**	**102, 729**
C. baileyi	Chicken	826	**254, 572**	102, **104, 620**
C. felis	Cat	864	15, 33, **390, 426**	102, **104, 182, 476**
C. meleagridis	Turkey	833	11, 11, **108, 254, 449**	102, **104, 171, 456**
C. wrairi	Guinea pig	834	11, 11, **109, 254, 449**	102, **104, 628**
Cryptosporidium sp.	Desert monitor	834	19, 33, **109, 255, 418**	102, **104, 628**
C. parvum	Human	837	11, 12, **111, 254, 449**	**70**, 102, **104, 561**
C. parvum	Monkey	835	11, **109, 254, 461**	**70**, 102, **104, 559**
C. parvum	Bovine, A gene	834	11, 12, **108, 254, 449**	102, **104, 628**
C. parvum	Bovine, B gene	831	9, **119, 254, 449**	102, **104, 625**
C. parvum	Mouse	838	11, 12, **112, 254, 449**	102, **104, 175, 457**
C. parvum	Dog	829	20, 33, **105, 254, 417**	94, **102, 633**
C. parvum	Ferret	837	11, 12, **111, 254, 449**	102, **104, 174, 457**
C. parvum	Pig	838	9, 11, **365, 453**	102, **104, 632**
C. parvum	Kangaroo, koala	837	33, **109, 254, 441**	102, **104, 631**

[a] Numbers in bold are the sizes of bands visible on the electrophoresis gel.

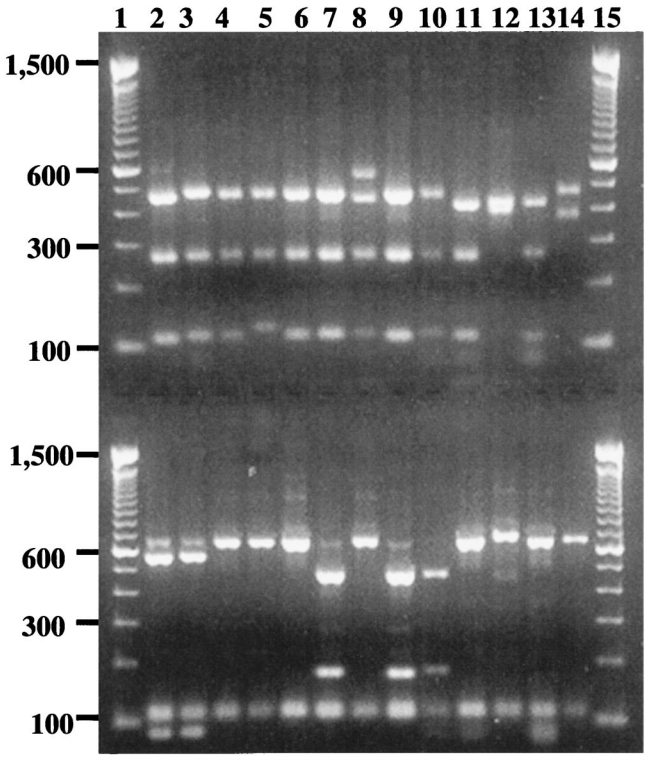

FIG. 2. Genotyping of the *C. parvum* group parasites by a nested PCR-RFLP procedure based on the SSU rRNA gene sequences. Lanes 1 and 15, molecular size markers; lane 2, *C. parvum* human genotype; lane 3, *C. parvum* monkey genotype; lane 4, *C. parvum* bovine genotype, A gene; lane 5, *C. parvum* bovine genotype, B gene; lane 6, *C. wrairi*; lane 7, *C. parvum* ferret genotype; lane 8, *C. parvum* kangaroo genotype; lane 9, *C. meleagridis*; lane 10, *C. parvum* mouse genotype; lane 11, *Cryptosporidium* sp. from a desert monitor; lane 12, *C. felis*; lane 13, *C. parvum* dog genotype; lane 14, *C. parvum* pig genotype. The upper lanes show *Ssp*I digestion products, and the lower lanes show *Vsp*I digestion products. Only partial digestion could be obtained with the kangaroo isolate by *Ssp*I and the *C. felis* isolate by *Vsp*I.

sporidium species considered to be valid by biologic characteristics, including *C. meleagridis*, *C. felis*, and *C. wrairi*, cluster together or within different *C. parvum* isolates. Their separate species status may need to be reexamined. Although *C. felis* is within the broad *C. parvum* clade in phylogenetic analyses, it is genetically different from the majority of *C. parvum* genotypes to such an extent that it may indeed represent a valid species. The *Cryptosporidium* parasites in dogs and pigs are traditionally classified as *C. parvum* (15, 30, 33). They are, however, genetically distant from the majority of *C. parvum* isolates in this study, and they may be cryptic species, especially if *C. wrairi* and *C. meleagridis* retain species status. Despite strong bootstrap support for some of the groupings, the relationships within the groups containing *C. parvum* and closely related isolates may be better resolved by the use of other genes such as the rRNA internal transcribed spacers. Biologic studies in addition to other genetic characterizations of various isolates are apparently needed before the taxonomic status of members in the broad *C. parvum* group can be clarified.

The isolate from a desert monitor is also more related to *C. parvum* than to any other species. Previously, *C. parvum*-like parasites have been seen in reptiles, but these parasites were found to be genetically identical to the *C. parvum* murine genotype, presumably as a result of ingesting a *C. parvum*-infected prey (29). The desert monitor had been in captivity for at least 6 years (acquired as an adult in 1992), and feeder mice that were used as the major diet were found to be infected (2 of 10) with the murine genotype of *C. parvum*. In addition, the desert monitor was shedding a large number of oocysts. Because the oocysts from the desert monitor were genetically different from the *C. parvum* murine genotype normally seen in mice, it is unlikely that the oocysts were from ingested prey. Recently, a new *Cryptosporidium* species, *C. saurophilum*, has been described from lizards, Schneider's skink (*Eumeces schneideri*), and desert monitors. The new species differs from *C. serpentis* by having smaller oocysts, developing in the intestine, and an inability to infect snakes (23). It is unclear whether the oocysts from the desert monitor in the present study also belong to the same new *Cryptosporidium* species. Genetic characterization of *C. saurophilum* is in progress by the original researchers and will help address this issue.

Results of this study confirm the heterogeneity of *C. parvum*. In addition to the previously described human, bovine, pig, mouse, and kangaroo genotypes, three additional genotypes (dog, ferret, and monkey) have been characterized. To date, with the exception of the bovine genotypes (27), each of these genotypes occurs only in their respective hosts, suggesting that host specificity may exist among these genotypes. Limited cross-transmission studies with the human and murine genotypes confirm the existence of host specificity (22, 32). Differences in host specificity were used as the basis for the separation of *C. wrairi* and *C. felis* from *C. parvum*. If the same standard is used in species designation, many of the other isolates from the larger *C. parvum* group may be considered distinct species. Extensive biologic characterization is needed to address this issue.

The significance of this genetic diversity in the *C. parvum* group is not clear. Companion animals and rodents have been frequently suggested as a source of infection for humans and farm animals (4, 8, 10, 20, 22, 24, 43, 44). It has been recently suggested that all *Cryptosporidium* isolates, including those from lower vertebrates, should be considered as hazardous to humans (41). In view of the genetic heterogeneity and associated host specificity, this point of view needs to be reassessed, especially the infectivity of these parasites to immunocompetent humans. Thus far, only the human and bovine genotypes of *C. parvum* have been found in humans (3, 5–7, 28, 32, 36, 37, 39, 45, 47). A *C. baileyi*-like parasite was reported in a patient with AIDS (13), but the identity of this parasite has been subsequently questioned by the original investigators (12). Even though studies conducted to date have only identified the human and bovine genotypes of *C. parvum* in humans, further studies with larger sample sizes are needed to test if nonparvum *Cryptosporidium* spp. or other genotypes of *C. parvum* are infectious in humans, especially immunocompromised individuals. The use of PCR and sequencing tools as shown in this study would make these studies possible.

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